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Bacteria associated with the hepatopancreas of the woodlice *Oniscus asellus* and *Porcellio scaber* (Crustacea, Isopoda)

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With 6 figures

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1. Introduction

The hepatopancreas of woodlice is thought to perform functions in digestion and absorption (McMurrich 1898; Hartenstein 1964), in the secretion of enzymes (Clifford & Witkus 1971; Hassall & Jennings 1975) and in the storage of metabolic reserves (Szyfrer 1966), copper and other heavy metals (Hopkin & Martin 1982). Microorganisms have been reported to be associated with the microvillous border in the hepatopancreas of the terrestrial isopods *Porcellio dilatus* Brandt [Donadey & Besse 1972] and *Oniscus asellus* L. [Hopkins & Martin 1982]. The role of these microorganisms in their hosts was not discussed, although the isopod *Philoscia muscorum* (Scopoli) was thought to utilize cellulase produced by microorganisms in the gut (Hassall & Jennings 1975). In this paper conditions in the hepatopancreas and the distribution and morphology of the bacteria in the two species are compared.

2. Materials and methods

2.1. Preparation and *p*H range of hepatopancreas lobes

Specimens of the woodlice *O. asellus* L. and *P. scaber* Latr. were collected from beech (*Fagus sylvatica* L.) woodland at the Macaulay Institute. The hepatopancreas lobes and hind-gut were removed as previously reported (Griffiths & Wood 1985). The *p*H range was determined by macerating the lobes in the presence of solid *p*H indicators (bromocresol green; bromocresol purple; bromothymol blue; cresol red; methyl red and phenol red).

2.2. Determination of particle size excluded from the hepatopancreas

The size range of particles entering the hepatopancreas was determined by feeding recently fallen beech leaves coated with either charcoal (2.5—25.0 μm diameter), spores of the fungus *Aspergillus niger* van Tieghem (1.5—5.0 μm diameter) or fluoresbrite carboxylate microspheres (1.17 μm diameter, Polysciences Ltd., Northampton) to woodlice in plastic specimen jars with a base of moist "plaster of paris". After 4 d the hindgut, hepatopancreas and faeces were examined microscopically for the presence of particles. The fluorescent fluoresbrite microspheres were observed using a Zeiss Universal microscope equipped with a HBO 200 mercury vapour lamp, using exciter filter BG3 (transmission range 270—480 nm) for UV illumination and barrier filter 50/44 to observe light over 500 nm.

2.3. The morphology and distribution of bacteria in the hepatopancreas

Approximately 100 specimens of the woodlice were collected from Macaulay Institute soil (Grid Ref. NJ905048), garden compost at Mill-Hill, London (G.R. TQ 205918) and a mixed deciduous woodland, Moore's Wood, London (G.R. TQ560966). Hepatopancreas lobes smeared onto microscope slides and heat-fixed were stained for gram and acid fast reaction (Harrigan & McCance 1966). The occurrence and morphology of the bacteria were observed on the gram stained smears by light

microscopy and their lengths were determined using a Vids II two dot measurement system (Analytical Measuring Systems Ltd., Saffron Walden). Statistical differences between the mean lengths of bacteria from different sites were determined by a *t*-test.

For electron microscopy, hepatopancreas lobes were fixed in 2.5% v/v glutaraldehyde in 0.1 M sodium cacodylate buffer *p*H 5.4, at room temperature for 1 h and then washed in buffer. Samples for scanning electron microscopy were prepared by the method of NATION (1983). The lobes were attached to aluminium stubs using colloidal carbon, fractured using a scalpel, coated with gold and examined in a Cambridge S4 stereoscan. Specimens for transmission electron microscopy were post fixed in 1% osmium tetraoxide in buffer at 4 °C overnight. The lobes were then dehydrated, embedded in Agar 100 resin (Agar Aids Ltd., Stansted) and polymerised at 60 °C (GLAUERT 1975). Transverse sections cut on a Reichert Ultramicrotome OMU3 (Reichert-Jung UK, Slough), using a diamond knife, were stained with uranyl acetate and lead citrate (KNIGHT 1977) and examined in a Siemens Elmiskop 102 transmission electron microscope at 80 kv.

2.4. Isolation of bacteria

Varous isolation media and incubation conditions were used in attempts to isolate the bacteria. The media included different mineral salts with additions such as glucose, soil extract and vitamins (OWENS & KEDDIE 1969), media for *Kurthia* (GARDENER 1969), *Lactobacilli* (ROGOSA *et al.* 1951) and actinomycetes (KÜSTER & WILLIAMS 1964; NONOMURA & OHARA 1971), egg yolk medium and tryptone soya agar (Oxoid). All media were used at *p*H 5.4 and 7.2. The hepatopancreas loves were removed aseptically and homogenised in 1 ml sterile water in a tissue grinder. Aliquots were either spread or streaked onto the surface of the agars and incubated under aerobic, elevated CO_2 concentration (10%) or anaerobic conditions (using anaerobic gas generating kits, Oxid) at 15 °C. The plates were inspected for growth after three weeks.

3. Results

3.1. *p*H and particle exclusion

The hepatopancreas of *O. asellus* and *P. scaber* had *p*H ranges of 4.7 to 5.4 and 5.4 to 6.1 respectively. Hindguts had the same *p*H range as the hepatopancreas. All the particles used to coat the leaves were present in the hindgut and faeces after 4 d, but were not observed in the hepatopancreas. Particles of 1.17 μm diameter and greater were, therefore, excluded from the hepatopancreas lobes.

3.2. Morphology of bacteria

All the bacteria observed in both isopods were gram positive and non-acid fast rods. In *O. asellus* the bacteria had a mean length of 3.2 $\pm$ 2.2 μm but occasionally attained lengths of 10 to 15 μm and rarely 37.5 μm (table 1). The majority of bacteria were shorter straight rods with rounded ends. In *P. scaber* the bacteria were similar morphologically but signifi-

Table 1. The frequency of woodlice containing bacteria in the hepatopancreas, and the length (μm) of those bacteria, in *Oniscus asellus* and *Porcellio scaber* collected from different sites

	Location and date				
	P. scaber	*O. asellus*			
	MISR*) (Sept)	MISR (Sept)	MISR (May)	Mill-Hill (May)	Moore's Wood (May)
No. of length measurements	372	1,646	698	699	2,297
Mean	2.00	3.20[b])	2.32[a])	2.52[a])	2.65[b])
S.E.	1.05	2.20	0.80	1.04	0.87
Minimum	0.54	0.49	0.63	0.91	0.57
Maximum	12.20	37.52	7.95	10.41	13.28
Total no. individuals	55	94	90	91	81
% with bacteria	21.8	46.8	8.9	7.7	28.4

*) Macaulay Institute for Soil Research

[a], [b]) Means with the same letter are not significantly different ($P \leq 0.05$).

cantly smaller ($P \leq 0.001$), with a mean length of $2.0 \pm 1.05\,\mu m$, occasionally being over $5\,\mu m$ and rarely $12.0\,\mu m$ (table 1). Although several long filaments were observed there was no evidence of branching. The bacteria in both species had a diameter of approximately $0.3\,\mu m$.

The bacteria were associated with the microvillous layer of the hepatopancreas cells in both species (fig. 1 and 2). They did not appear to be attached to the microvilli by any appendages, and flagella were not observed. Multiplication appears to be by transverse fission although in *O. asellus* it may not be binary. Transverse sections of the hepatopancreas revealed that the bacteria were distributed in the lumen between the cells (figs. 3 and 4). The cell wall of the bacteria in *O. asellus* (approximately 30 nm) was thicker than in *P. scaber* (approximately 10 nm) (figs. 5 and 6).

3.3. Occurrence of bacteria in woodlice from different sites

Not all of the specimens examined contained bacteria in the hepatopancreas (table 1). There was no difference between the frequency of occurrence in gravid females, non-gravid females or males in the specimens examined. There were, however, large differences in the frequency of positive specimens from different sites (table 1). At the Macaulay Institute there was also a large increase in positive *O. asellus* in September compared with May. The lengths of the bacteria in woodlice from the different locations also tendend to be significantly different (table 1).

3.4. Isolation of bacteria

There was no growth on the isolation media, except for colonies thought to be contaminants since they could not be isolated consistently.

4. Discussion

McMurrich (1898) suggested that digestion and absorption of nutrients, normal function of a midgut, were performed by the hepatopancreas in woodlice. However, Hartenstein (1964) stated that the role of the hepatopancreas was purely secretory and the region of straight line digestive tract immediately posterior to the opening of the hepatopancreas served as the absorptive midgut. Food particles were not observed in the hepatopancreas of *P. muscorum* (Hassall 1977), being prevented from entering by a filter system. A similar filter system may have prevented the particles of $1.17\,\mu m$ diameter or greater from entering the hepatopancreas lobes of *O. asellus* and *P. scaber*. The exclusion of particles suggests that the hepatopancreas does not function as a digestive caeca.

Electron and light microscope studies both indicated that there were very few different types of microorganisms present in the hepatopancreas. Particle exclusion may explain this low diversity. The hindgut of *O. asellus* contains 10^5—10^6 plateable bacteria $\times$ mg^{-1} dry mass (Ineson & Anderson 1985; Griffiths & Wood 1985), yet none could be reliably isolated from the hepatopancreas. This is unlikely to be due to a difference in *p*H as both the hindgut and hepatopancreas were acidic, which has also been demonstrated by Hartenstein (1964). Considering the lack of particles and other microorganisms and that bacteria could not be isolated on media covering a range of growth requirements, bacteria in the hepatopancreas may be extracellular obligate endosymbionts in the sense of Dasch *et al.* (1984). Isolation of other endosymbiotic bacteria from various arthropods has been recorded by several workers, although the claims have not been substantiated (Brooks 1970).

The bacteria in the hepatopancreas of *O. asellus* and *P. scaber* are probably different, as indicated by difference in their size (Table 1) and cell wall structure. The cell wall of bacteria from *O. asellus* was typically gram positive with an electron dense layer approximately 30 nm wide (fig. 5). The cell wall of bacteria from *P. scaber* also appeared to be gram positive although the electron dense layer was narrower (fig. 6). The extracellular bacteria-like endosymbionts of Hemiptera (Dasch *et al.* 1984) and the intercellular endosymbionts in

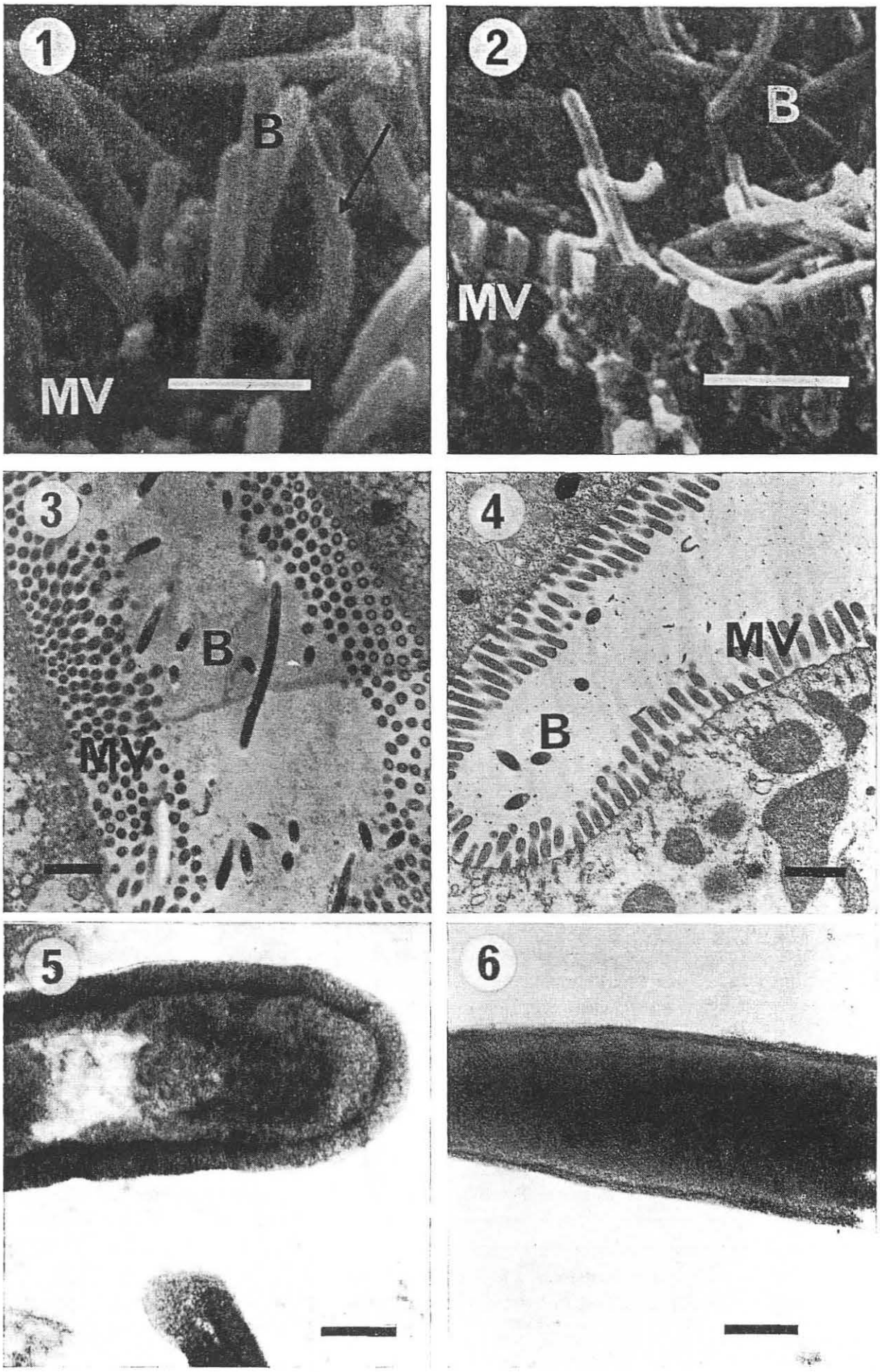

Fig. 1—6

Homoptera (Houk & Griffiths 1980) and the woodant (Lamparter 1967) were gram negative. Although endosymbionts in cockroaches were gram positive they had a typical gram negative cell wall, and dilute sodium dodecyl sulphate abolished the gram positive staining property (Malke & Bartsch 1966).

Arthropods harbour microorganisms supposedly beneficial to the host, but it is rarely found possible to establish the beneficial effect beyond doubt (Brecher & Wigglesworth 1944; Pant & Fraenkel 1950). Brooks (1970) suggested that endosymbionts may be encouraged to grow when the host is feeding upon nutritionally poor substrates, and that they provide a supplement such as vitamins or enzymes to hydrolyse more complex material. If the bacteria were present in only nutritionally stressed organisms, this may account for the fact that they were not found in all specimens examined and for the variation in occurrence between sites and collection dates. The effects of such large numbers of bacteria in the hepatopancreas will, however, be difficult to assess until they can be isolated.

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Figs. 1 and 2. Scanning electron micrograph of the internal surface of hepatopancreas from *O. asellus* (1) and *P. scaber* (2). Bacteria (B) are visible on the microvillous layer (MV) and some bacteria can be seen dividing (arrowed). Bar, 2 μm.

Figs. 3 and 4. Transmission electron micrograph of transverse section through the hepatopancreas of *O. asellus* (3) and *P. scaber* (4). Bacteria (B) are distributed in the lumen of the hepatopancreas between the microvillous layers (MV) of the cells. Bar, 1 μm.

Figs. 5 and 6. Transmission electron micrograph showing the cell wall structure of bacteria from the hepatopancreas of *O. asellus* (5) and *P. scaber* (6). Bar, 0.1 μm.

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Bacteria associated with the hepatopancreas of the woodlice *Oniscus asellus* L. and *Porcellio scaber* Latr. are gram positive and non-acid fast rods. They occasionally attain lengths up to 37.5 μm, although the majority are much shorter having a mean length of 3.20 and 2.00 μm, respectively, and a diameter of 0.3 μm. Bacteria are associated with the microvillous layers of the hepatopancreas cells. Not all the individual woodlice examined possessed bacteria and there was a considerable difference in occurrence between species, collection sites and collection dates. It was not possible in this study to isolate the bacteria.
Key words: Woodlice, *Oniscus asellus*, *Porcellio scaber*, gutflora, hepatopancreas, endosymbiont.